Characterizing the Essential Roles of Histone Acetyl Transferase 1 during Epigenetic Inheritance in a Mammalian Model

Undergraduate Honors Thesis

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Acknowledgments

I'd like to thank my mentor and advisor, Dr. Mark Parthun, for the opportunity to join his laboratory and for his encouragement and enthusiasm for my own contributions to his research. I'd also like to thank the other members of my laboratory, Dr. Prabakaran Nagarajan, Paula Agudelo-Garcia, and Pei Zhang for welcoming me into their laboratory and for helping me through this incredible learning experience. Additionally, I would also like to thank the College of Arts and Sciences for funding my undergraduate research scholarship.

Abstract

Hat1 is a histone acetyltransferase responsible for an evolutionarily conserved diacetylation of newly synthesized histone H4 at lysines 5 and 12 and is required for the proper acetylation of newly synthesized histone H3 at lysines 9 and 27. Hat1 is essential for viability in mice. Complete deletion of murine Hat1 ($Hat1^{-/-}$) is neonatal lethal, resulting in developmental defects in the embryonic lung. Hat1 has long been believed to play a role in epigenetic inheritance, however evidence to support this hypothesis has remained elusive and a role for this pattern of histone acetylation during mammalian development has been largely uncharacterized. By analyzing the chromatin architecture of $Hat1^{-/-}$ mouse embryonic fibroblasts, we discovered 853 loci-specific peaks of H4K5 acetylation dependent upon the presence of Hat1 that are also necessary for proper gene activation. Consistent with this observation, an in vivo transcriptome study of E17.5 $Hat1^{-/-}$ embryonic lungs demonstrated 172 RNA transcripts found to be under-expressed at least 2-fold or greater when compared to the wild-type. Furthermore, the down-regulation of Sftpa1 and Cyp-2f2 suggests that the developmental defects observed in the lungs of $Hat1^{-/-}$ mice may be indicative of defects in the cellular differentiation of alveolar and Clara cell precursors. Additionally, mice containing a liver-specific knockout of Hat1 demonstrate a high incidence of rare liver cancer types, such as hepatoblastoma and hepatocholangiocellular carcinoma, that are known to arise from early hepatocyte precursors. Together, our findings suggest a role for Hat1 as an epigenetic regulator involved in the appropriate activation of gene required for cellular differentiation from stem cell-like cellular precursors.

Introduction

In eukaryotes, DNA is wrapped around an octamer of histone proteins that are tightly packaged into a structure known as chromatin [1]. Modifications to the tails of these histone proteins may alter and distort normal chromatin structure that can regulate whether or not a region of DNA is accessible to transcriptional machinery. Depending on the type of histone modification made, these cell-specific modifications on the chromatin architecture provide a mechanism for differential gene expression [1]. This process, known as epigenetic regulation, is what allows genetically identical cells of a multicellular organism to present with great structural and functional diversity.

Histones, which are synthesized in the cytosol, must go through a highly structured process of histone modification prior to their incorporation into the chromatin architecture in order to accurately maintain or modify the epigenetic landscape [1]. Histone acetyltransferase 1 (Hat1) is of particular interest because it is one of a select number of type B histone acetyltransferases, which are capable of acetylating newly synthesized and unmodified cytoplasmic histones [2]. Specifically, Hat1 functions as the sole enzyme responsible for an evolutionarily conserved diacetylation of newly synthesized histone H4 at lysines 5 and 12 and is also required for the acetylation of newly synethesized histone H3 at lysines 9 and 27 [3]. During chromatin assembly, these modified histones are imported into the nucleus and incorporated into the chromatin in either a replication-coupled or replication-independent fashion (Figure 1). However, a role for this conserved initial pattern of histone diacetylation has been largely uncharacterized. Moreover, very little is known about how patterns of initial histone acetylation prior to chromatin assembly influence epigenetic inheritance. Understanding why newly synthesized histones are primed by Hat1 prior to chromatin assembly is crucial to understanding the mechanisms by which epigenetic inheritance takes place.

In the past, a substantial amount of work has been put forth to characterize a role for Hat1-specific histone acetylation during chromatin assembly. Early studies of Hat1 were primarily worked out in a

yeast model organism. Evidence from studies in yeast has implicated Hat1 in DNA double-strand break repair and telomeric silencing pathways [4, 5]. However, loss of Hat1 in yeast shows no influence on chromatin assembly or cell viability, and yeast containing a complete deletion of Hat1 display no differences in gene expression or phenotypes indicative of defects in epigenetic inheritance. Due to the high degree of conservation of Hat1 histone acetylation and the potential for functional redundancies of Hat1 in yeast, recent studies utilized a mouse model of Hat1 in order to further understand its influence over chromatin assembly and epigenetic inheritance [2].

Studies of murine Hat1 began following the generation of a conditional Hat1 knockout mouse model (Figure 2) [3]. Consistent with yeast, *in vitro* studies in mouse embryonic fibroblasts (MEFs) have indicated that Hat1 is necessary for appropriate genome stability. Complete deletion of Hat1 ($Hat1^{-/-}$) results in a higher frequency of chromosomal abnormalities and numerous γ-H2AX foci in the absence of DNA damaging agents, which are hallmarks of genome instability [3]. Though unlike yeast, murine Hat1 is essential for viability. Loss of Hat1 is neonatal lethal, and $Hat1^{-/-}$ mice die either in gestation or shortly after birth (Figure 3) [3]. The neonatal lethality of $Hat1^{-/-}$ mice is due to a thickening of the lung mesenchyme, resulting in constriction of lung alveoli and subsequent respiratory failure (Figure 4) [3]. Ki67 staining has demonstrated that the developmental defects observed in the lungs of $Hat1^{-/-}$ neonates are due to a hyperproliferation of the lung mesenchyme (Figure 4c) [3]. Furthermore, cleaved Caspsases 3 staining has shown no abnormalities in apoptosis, which is consistent with a hyperproliferative phenotype (Figure 4d).

While mice heterozygous for Hat1 ($Hat1^{+/-}$) are viable, there is a slight decrease in the number of expected $Hat1^{+/-}$ pups that are obtained from heterozygous crosses (Figure 3) [3]. After their first year, $Hat1^{+/-}$ mice begin to show a marked decrease in survival with a median survival of 80 weeks (Figure 5a). Interestingly, autopsies of naturally deceased $Hat^{+/-}$ mice suggest that they may be particularly prone to liver-specific cancers (Figure 5b). The developmental defects observed in the lung and liver strongly suggest that Hat1 plays an essential role during epigenetic inheritance and mammalian development. To

delineate a potential mechanism by which Hat1 may direct the appropriate transmission of epigenetic information, we have utilized both *in vitro* and *in vivo* studies of murine Hat1 to further characterize its roles in mammalian development.

Methodology

Generation of Liver-Specific Knockout Mice

To generate a liver-specific deletion of Hat1 in mice, $Hat1^{+/-}$ mice were crossed with Alb-Cre transgenic mice to give rise to $Hat1^{+/-/Alb\text{-}cre}$ transgenic mice. $Hat1^{+/-/Alb\text{-}cre}$ transgenic mice were subsequently crossed with $Hat1^{Flox/Flox}$ transgenic mice to produce $Hat1^{Flox/-/Alb\text{-}cre}$ transgenic mice. Genomic DNA was extracted by standard methods using pheno: chloroform isolation and genotypes were determined by PCR.

Primers used to detect $Hat1^{+}$ and $Hat1^{-}$ alleles were P1: 5′-GCC TGG TGA GAT GGC TTA AAC -3′ and P2: 5′-GCA AGT AGT ATG ACA AGA GGT AGG -3′. PCR was performed under the following conditions; 95°C for 50 min followed by 29 cycles at 95°C for 30 sec., 54.6°C for 30 sec. and 72°C for 80 sec. and final extension for 5 min. at 72°C.

Primers used to detect the $Hat1^{Flox}$ allele were P1: 5'-GCT CAG TGG TCA CAA GTT AGAT-3' and P2: 5'-AGC TGA TGG GTA TAT TCA GGA AAG-3'. PCR was performed under the following conditions; 95°C for 50 min followed by 29 cycles at 95°C for 30 sec., 56°C for 30 sec. and 72°C for 30 sec. and final extension for 5 min. at 72°C.

Primers used to detect the Alb-Cre alleles were P1: 5'-CCT GTT TTG CAC GTT CAC CG-3' and P2: 5'-ATG CTT CTG TCC GTT TGC CG-3'. PCR was performed under following the conditions; 95°C for 50 min followed by 29 cycles at 95°C for 1 min, 57.4°C for 1 min and 72°C for 1 min and final extension for 5 min. at 72°C.

All animal use was performed according to the guidelines of The Ohio State University Institutional Animal Care and Use Committee (IACUC) under permit number 2007A0094R2

Preparation of Liver tissues

After 80-weeks, whole livers were excised from adult mice by standard dissection. Liver tissues were fixed in 20 mL of 10% Neutral Buffered Formalin solution for 48 hours. Following fixation, tissues were stored in 70% ethanol. Immunohistochemistry was performed by standard procedures. Hematoxylin/Eosin and PAS staining were performed with staining kits from DAKO.

Quantitative Real-Time PCR

RNA was extracted from $Hat1^{+/+}$ and $Hat1^{-/-}$ MEF cell lines with trizol using standard extraction protocols from Invitrogen. RNA was converted to cDNA using standard procedures. cDNA was diluted to 10 ng/µl. All quantitative real-time PCR reactions were performed and analyzed using 7300 system SDS software. Amplifications were performed using TaqMan Gene Expression Master Mix protocols from Applied Biosystems. The thermal cycling conditions were composed of 50°C for 2 min, followed by an initial denaturation step at 95°C for 10 min, 40 cycles at 95°C for 15 sec. and 60°C for 1 min.

Microarray

Female Hat1$^{+/-}$ mice were mated overnight with male Hat1$^{+/-}$ mice. If a vaginal plug was found the following morning, that day was counted as E0.5. At E17.5, pregnant females were euthanized and embryos were removed by cesarean section. Lung tissue was excised from embryos in cold PBS and stored at -80°C. Head and brain tissue were excised from embryos and used to genotype for the Hat1$^{+/+}$ and Hat1$^{-/-}$ embryos using previous described genomic DNA extraction and PCR protocols.

Total RNA was extracted from 10mg of embryonic lung tissue using the Animal Tissue RNA Purification Kit (Norgen) following the manufacturers protocols. Total RNA quality was analyzed by Agilent software. Gene expression profiling for lung tissue from three wild-type and three knockout embryos was performed on separate mouse transcriptome array 1.0 microarrays (Affymatrix) in conjunction with the Ohio State University Microarray Shared Resources. The transcriptome was analyzed using Ingenuity software.

Results

Hat1 influences global and local alterations to the chromatin architecture

Since Hat1 functions to acetylate newly synthesized histone H4 at lysine 5 (H4K5), we performed ChIP-Seq to target and map H4K5 acetylation patterns on the chromatin of $Hat1^{+/+}$ and $Hat1^{-/-}$ MEFs[1]. Globally, loss of Hat1 led to a uniform decrease in basal H4K5 acetylation across the genome (Figure 6a). Interestingly, Hat1 also appeared to influence local changes to the epigenetic landscape. We observed 853 loci-specific peaks of H4K5 acetylation missing from the $Hat1^{-/-}$ MEF chromatin that were either completely or partially-dependent on the presence of Hat1 (Figures 6b and 6c). Partially-dependent peaks were defined as those that displayed a >50% reduction in area.

Hat1 influences gene expression through loci-specific modifications to the chromatin architecture

To determine if the local changes to the chromatin architecture that was dependent upon the presence of Hat1 can influence gene expression, we performed quantitative real-time PCR directed at genes associated with loci-specific peaks in H4K5 acetylation missing from $Hat1^{-/-}$ chromatin. At around 0.75-1.2Mb upstream of Lphn3, we found peaks of H4K5 acetylation that were completely and partially-dependent upon the presence of Hat1 (Figures 6b and 6c). Since we observed multiple Hat1-specific peaks of acetylation upstream from Lphn3, we quantified Lphn3 mRNA to detect changes in the expression of Lphn3 in $Hat1^{-/-}$ MEFs. In the absence of these Hat1-dependent peaks of H4K5 acetylation, we observed a significant 3.5-fold decrease in the levels of Lphn3 mRNA in $Hat1^{-/-}$ MEFs when compared to those found in $Hat1^{+/+}$ MEFs (Figure 7).

Hat1 exerts epigenetic control over embryonic lung development

Since the developmental defects observed in the lungs of $Hat1^{-/-}$ neonates were completely penetrant, it was an ideal starting point for an *in vivo* study of whether or not Hat1 functions to influence epigenetic inheritance. Embryonic lung tissue excised from 3 $Hat1^{-/-}$ and 3 $Hat1^{+/+}$ embryos at E17.5 was processed for RNA and subjected to a microarray to characterize how Hat1 influences the transcriptome of the embryonic lung. Globally, we found 172 RNA transcripts that were down-regulated at least 2-fold or greater in $Hat1^{-/-}$ embryos when compared to their wild-type counterparts (Figure 8a). Conversely, we found only 19 RNA transcripts that had been up-regulated in the lungs $Hat1^{-/-}$ embryos, suggesting that Hat1 functions predominantly to activate gene expression (Figure 8a). Interestingly, genes associated with the immune system, such as eosinophilic, lymphocytic, and neutrophilic-associated genes, were some of the most highly under-expressed genes (Figure 8b). While a thorough analysis of the $Hat1^{-/-}$ lung transcriptome is still required, there were a number of genes whose expression profile is indicative of defects in cellular differentiation in the lung. For example Sftpa1, which is a marker for alveolar differentiation and maturation, was under-expressed 2.36-fold in $Hat1^{-/-}$ lung tissue (Figure 8b) [8]. Additionally Cyp-2f2, which is a marker for Clara cells differentiation and maturation, was found to be under-expressed 1.52-fold in $Hat1^{-/-}$ lung tissue (Figure 8b) [9].

Hat1 may function to ensure the appropriate maintenance of liver tissues

Since $Hat1^{+/-}$ adult mice are prone to liver-specific cancers, Hat1 may also play a role in the maintenance and appropriate development of the liver. To observe the influence of Hat1 in the liver, we generated liver-specific Hat1 knockout mice ($Hat1^{L-/-}$). While a liver-specific deletion of Hat1 was not essential for viability, $Hat1^{L-/-}$ mice displayed an accumulation of fat in their livers similar to that found in non-alcoholic fatty liver disease (Figure 9) [12]. Additionally, after a period of 80 weeks both $Hat1^{+/-}$ and $Hat1^{L-/-}$ mice demonstrated a high incidence of hepatocellular carcinoma, hepatoblastoma, and

hepatocholangiocellular carcinoma (Figures 10 and 11). Heptaoblastoma and hepatocholangiocellular carcinoma cancer types are particularly rare and originate from very early hepatocytic precursor (Figure 11) [10]. It should be noted that we are presently unable to provide pathologies for wild-type mice decedent from the same liver-specific Hat1 knockout colony as a control to our current study. As such, it is important to note that these tumors may have formed for reasons unrelated to Hat1, such as a Hat1-independent increased risk of liver cancer found within our liver-specific knockout colony. Nonetheless, these rare cancer types are not typically found in wild-type mice and have not been found in 80 week old wild-type mice from our other colonies of mice.

Discussions and Considerations[2]

Murine Hat1 is essential for viability, and the complete deletion of Hat1 in a mouse model results in developmental defects and neonatal lethality [3]. Since Hat1 functions in histone acetylation, this strongly suggests that Hat1 may play an essential role in epigenetic inheritance. Based off of our observations, there may be three (or more) potential reasons to explain the link between Hat1 and its influence over mammalian development.

First, that Hat1 histone acetylation is required to ensure appropriate genomic stability that would otherwise lead to neonatal lethality. In both yeast and mice, Hat1 has been shown to be necessary for the appropriate maintenance of the genome [2, 3]. Specifically, $Hat1^{-/-}$ MEFs fail to properly maintain genomic stability and demonstrate a higher susceptibility to endogenous DNA damage and chromosomal abnormalities [3]. An increased susceptibility to DNA damage may in part explain the observed decline in survival of $Hat1^{+/-}$ mice and the diversity of cancers we found in $Hat1^{+/-}$ and $Hat1^{L-/-}$ mice. However, it would be extremely unlikely that a susceptibility to DNA damage would consistently produce the developmental defects observed in the lungs of $Hat1^{-/-}$ neonates, suggesting that Hat1 functions to play other essential roles in mammalian development.

It may also be possible that Hat1 is capable of acetylating other cellular proteins other than histones that are required for appropriate mammalian development. Paula Agudelo-Garcia from the Parthun lab has demonstrated that Hat1 localizes to the mitochondria and is required for proper mitochondrial function and mitochondrial protein acetylation (Figure 12)[3]. This preliminary evidence may actually explain some of the defects observed in the liver, in which mitochondria are extremely abundant. If it is confirmed that Hat1 is in fact capable of directly acetylating mitochondrial proteins, then it would open up the possibility that Hat1 may be capable of acetylating other cellular proteins in addition to histones that would be necessary for appropriate mammalian development. In fact, it has been recently reported that Hat1 is responsible for acetylating the PLZF transcription factor, which

functions to moderate the production of inflammatory cytokines and NF-kB response pathways [6]. While activation of PLZF does not sufficiently explain the developmental defects observed in the Hat1$^{-/-}$ embryonic lung, it may explain certain observations such as the global down-regulation of genes associated with the immune system. Nonetheless, it may be possible that Hat1 acetylation of other non-histone proteins would sufficiently explain all developmental defects observed in the lungs of Hat1$^{-/-}$ neonates.

The third potential reason to explain the developmental defects we observed in Hat1$^{-/-}$ mice would be that Hat1 histone acetylation is required for the appropriate transmission and/or activation of epigenetic information during mammalian development. The global and loci-specific influences of H4K5 acetylation dependent upon the presence of Hat1 in conjunction with the real-time PCR expression profile for Lphn3 strongly suggest that Hat1 acts to selectively target and activate the expression of a specific subset of genes through histone modifications. Since this observation is consistent with the global down-regulation of genes found in the lung transcriptome of Hat1$^{-/-}$ embryos, it may be reasonable to assume that Hat1 histone acetylation plays a role in epigenetic regulation and that Hat1-mediated gene activation is responsible for some of the developmental defects observed in the Hat1$^{-/-}$ neonates.

These roles for Hat1 are not necessarily mutually exclusive, and may very well all be required functions for appropriate mammalian development. And while this research does lend support to a role for Hat1 in gene activation, it lends little knowledge to the mechanisms by which Hat1 directs its actions. Since epigenetic inheritance and regulation is a complex and highly coordinated process, it would be unlikely that Hat1 histone acetylation would function alone to orchestrate these patterns of specific gene activation [1]. For this reason, we have chosen to propose a model in which Hat1 histone acetylation, specifically at H3K9 and H3K27, acts to prevent rapid and repressive PcG-mediated trimethylation of H3K27 during chromatin assembly following the incorporation of a new nucleosome onto nascent DNA (Figure 13). In doing so, Hat1 would provide the cell with a window of opportunity to activate genes previously silenced by repressive histone trimethylation during a period of chromatin maturation.

Following chromatin maturation, Hat1 specific histone modifications can be removed by HDACs to allow for the re-establishment of repressive trimethylation (Figure 13).

The Polycomb Group protein complex (PcG) functions to re-establish transcriptional repression by trimethylating H3K27 at specific regions of DNA known as Polycomb Response Elements (PREs) following the replication of DNA [7]. Recently it was reported that during chromatin assembly in the S-phase, newly formed PRE regions remain unmethylated until well after the S-phase despite the fact that PcG complexes remain closely associated with the region [7]. In our model, Hat1-mediated H3K9 and H3K27 acetylation blocks the PcG complex from rapidly re-methylating histones at PREs (Figure 13). If this were true, Hat1 would provide a mechanism for cells to either re-establish PcG-mediated gene silencing by removing the histone modification or to activate genes near a PRE by introducing novel histone modifications that would permanently inhibit PcG methylation. This model is consistent with our research, as we would expect to see a large scale down-regulation of genes in the lungs of $Hat1^{-/-}$ embryos due to the fact that they would no longer be able to prevent rapid and repressive trimethylation at PREs (Figure 8a).

Interestingly, the PcG protein complex does not induce universal gene repression; rather it is restricted to repress only a certain subset of genes, often including a number of developmental regulators that induce cellular differentiation [8]. Thus, our model proposes a role for Hat1 to prime unmodified histones that inhibit the actions of PcG-mediated repression of differentiation genes such that precursor cells may have an opportunity to activate genes required for cellular differentiation. *In vivo* studies of the lung and liver have provided evidence to suggest that the observed abnormalities in $Hat1^{-/-}$ mice are indicative of defects in cellular differentiation. While the differentiation patterns of lung cell types in the embryonic lung have not been well characterized, the down-regulation of Sftpa1 observed in $Hat1^{-/-}$ embryonic lungs has been implicated as a marker for differentiated alveolar cells [9]. Moreover, weak expression of Cyp-2f2 has been demonstrated as one of the few markers for Clara progenitor cells [10]. Thus, the down-regulation of Sftpa1 and Cyp-2f2 may be indicative of an abnormally high population of

undifferentiated alveolar and Clara progenitor cells. In the liver, the high incidence of hepatoblastoma and hepatocholangiocellular carcinoma in liver-specific $Hat1^{+/-}$ and $Hat1^{L-/-}$ mice also suggests a defect in differentiation. Both hepatoblastoma and hepatocholangiocelular carcinoma cancers originate from immature hepatocytic precursors [11]. As previously mentioned, we have no wild-type mice from the liver-specific Hat1 knockout colony to compare our results with as a control. Nonetheless, the onset of these rare cancer types may be explained in part by a requirement of Hat1 to provide hepatocytic precursors with the opportunity to appropriately differentiate.

While this investigation proposes an essential epigenetic role for Hat1 during mammalian development, other potential roles for Hat1 during mammalian development, such as genomic stability and non-histone acetylation, are not mutually exclusive and may very well all be required for viability and development. While more research is required to prove many aspects of our model, future ChIP-Seq studies analyzing how the patterns of H3K9, H3K27, H4K5, and H4K12 acetylation compare to and overlap with patterns of PcG trimethylation in the $Hat1^{-/-}$ cells, in conjunction with a further characterization of the embryonic lung and liver transcriptome, should be particularly interesting.

Figures

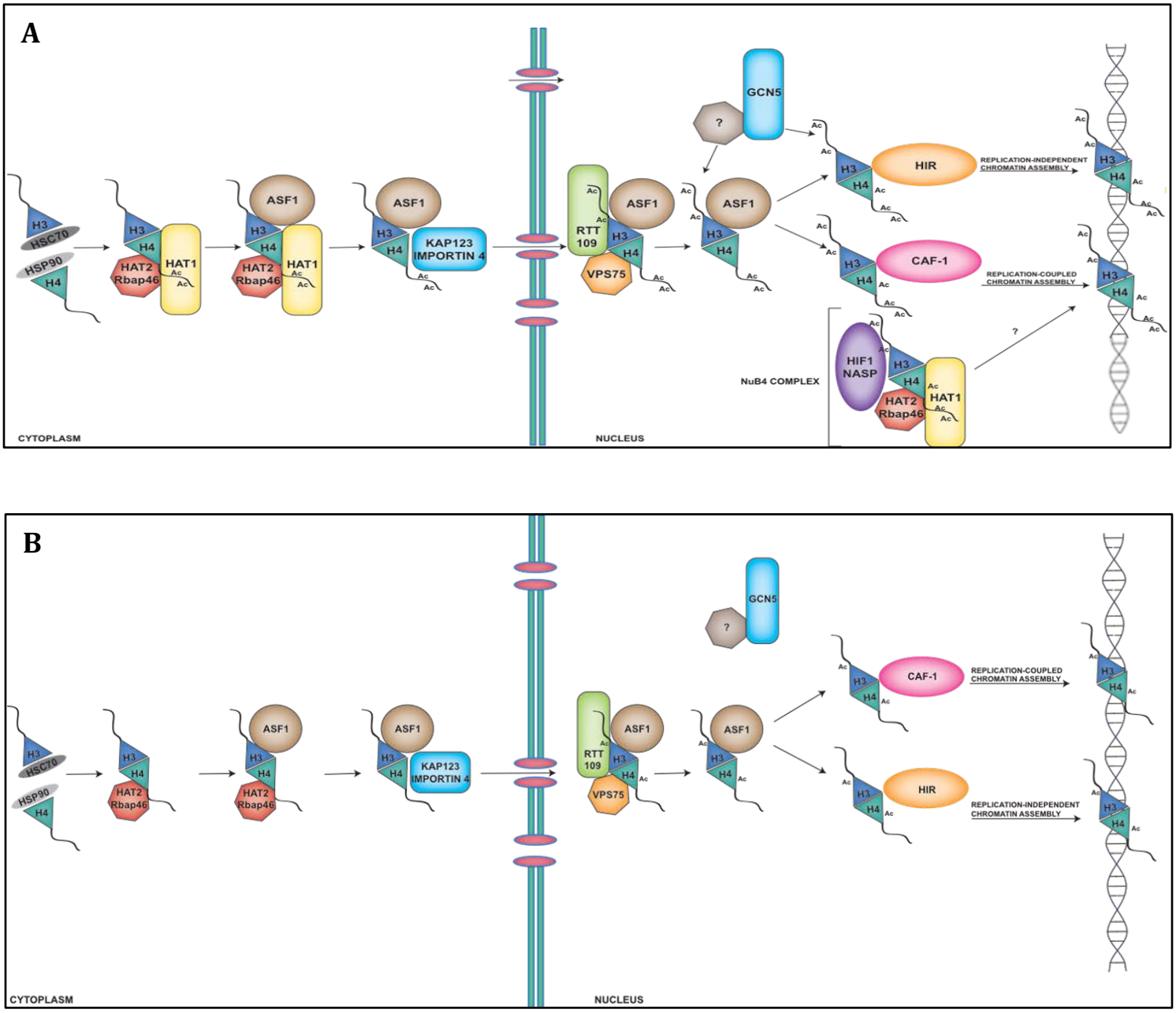


Figure 1: Systematic diagram of the current model for the processing of newly synthesized histones H3 and H4 during chromatin assembly

A) In the presence of Hat1, Hat1 acetylates newly synthesized cytoplasmic histones H3 at lysines 9 and 27 and H4 at lysines 4 and 12. These histones are transported into the nucleus and used for replication-coupled or replication-independent chromatin assembly. B) In the absence of Hat1, lysines 9 and 27 on histone H3 and lysines 4 and 12 on histone H4 are loaded onto the chromatin in an unacetylated state.

Figures

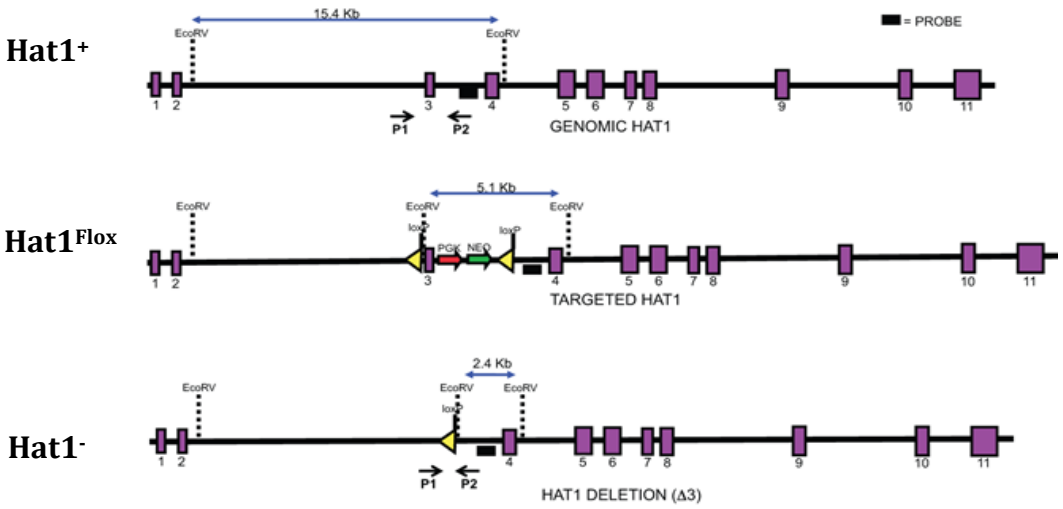


Figure 2. Diagram of the wild-type Hat1 locus (top), the Hat1 locus following the integration of loxP sequences flanking intron three (middle) and the Hat1 locus following Cre-mediated deletion of exon 3. Cre recombinase-mediated deletion of exon 3 results in the introduction of a stop codon and subsequently creates a truncated mutant of Hat1 [3].

A

B

HAT1$^{+/-}$ X HAT1$^{+/-}$ (36 MATINGS)				
GENOTYPE	**HAT1$^{+/+}$**	**HAT1$^{+/-}$**	**HAT1$^{-/-}$**	**TOTAL**
PUPS EXPECTED	77	154	77	308
PUPS OBTAINED	77	137	26	240
PUPS VIABLE	77	108	0	185

Figure 3. Hat1 is essential for viability in mice
A) Visual representation of neonatal pups [3]. B) Table listing the number of expected, obtained, and viable pups from 36 Hat1$^{+/-}$ matings. Hat1$^{-/-}$ pups are completely inviable and show a marked decrease in number obtained [3].

Figures

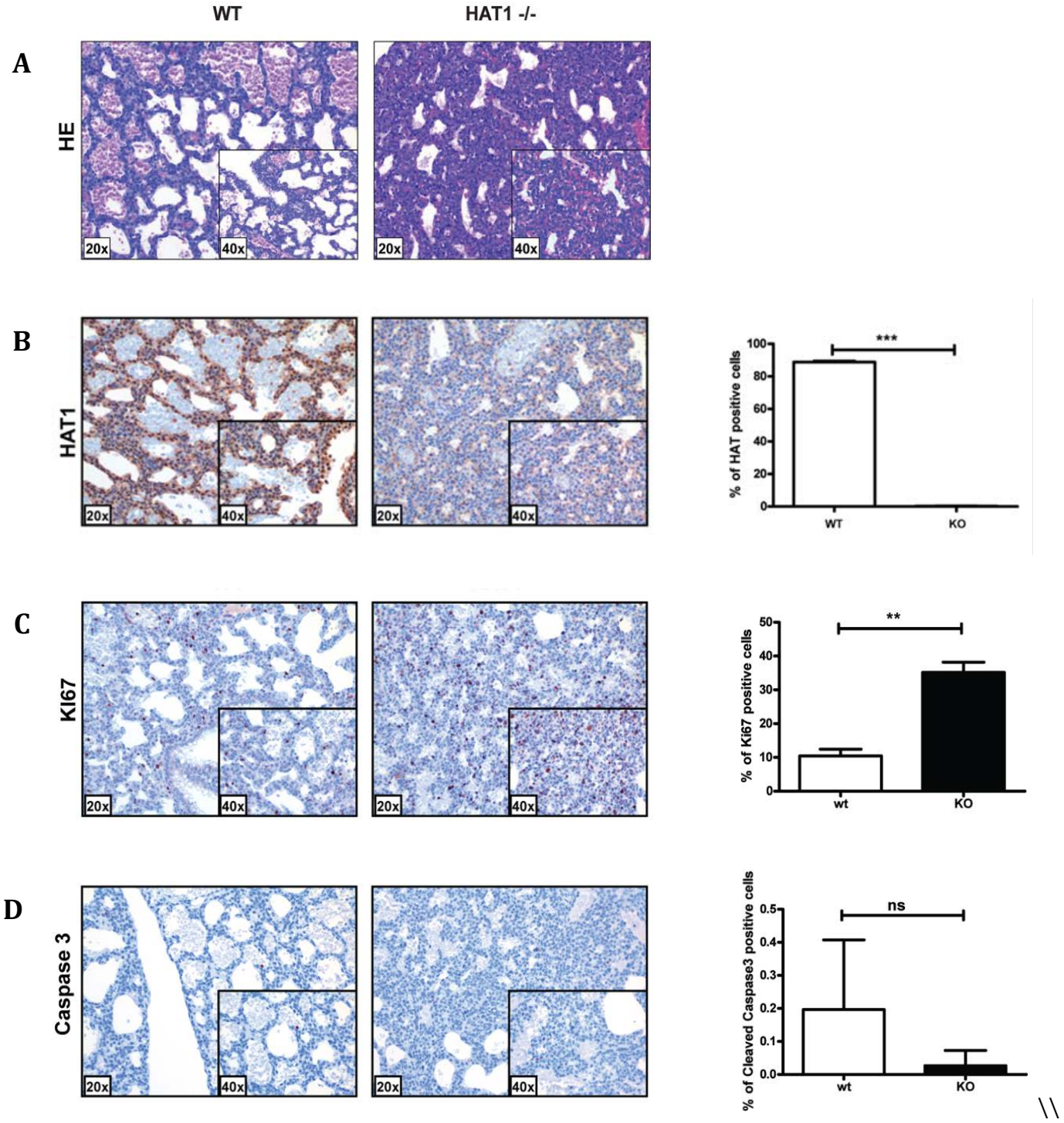
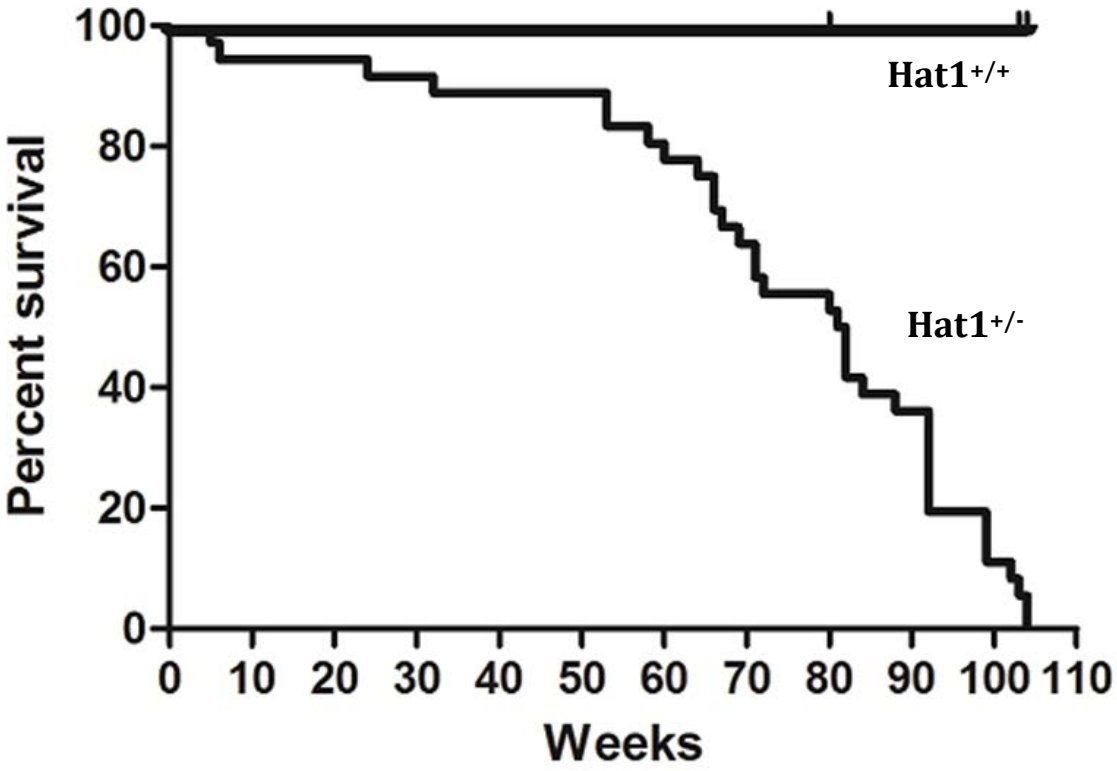


Figure 4. Developmental lung defects in the absence of Hat1.
Histology of lungs taken from newborn Hat1$^{+/+}$ (wild-type) and Hat1$^{-/-}$ pups at 20x magnification (inlets 40x) [3]. A) Staining with Hematoxylin-Eosin demonstrates a thickening of the lung mesenchyme. B) Hat1 is highly expressed in Hat1$^{+/+}$ pups, but not in Hat1$^{-/-}$ pups. C) KI67 stained by IHC shows significantly higher proliferation rates in the lungs of Hat1$^{-/-}$ pups. D) Cleaved Caspase3 stained by IHC showed no significant difference in apoptosis in the lungs of Hat1$^{-/-}$ pups.

Figures

A



B

TUMOR SITE	% OF ANIMALS (n=22)
LIVER	45
SPLEEN	32
INTESTINE	18
THYMUS	5

Figure 5. After 1 year, Hat1$^{+/-}$ mice display a steady decline in survival with a high incidence of liver cancers.
A) Kaplan-Meier plot for 38 Hat1$^{+/-}$ mice. After one year, there is a steady drop in the survival of Hat1$^{+/-}$ mice. Median survival occurs at around 80 weeks B) Tumors found upon visual inspection of 22 Hat1$^{+/-}$ mice that had been found dead. 45% of Hat1$^{+/-}$ mice demonstrated liver-specific cancers.

Figures

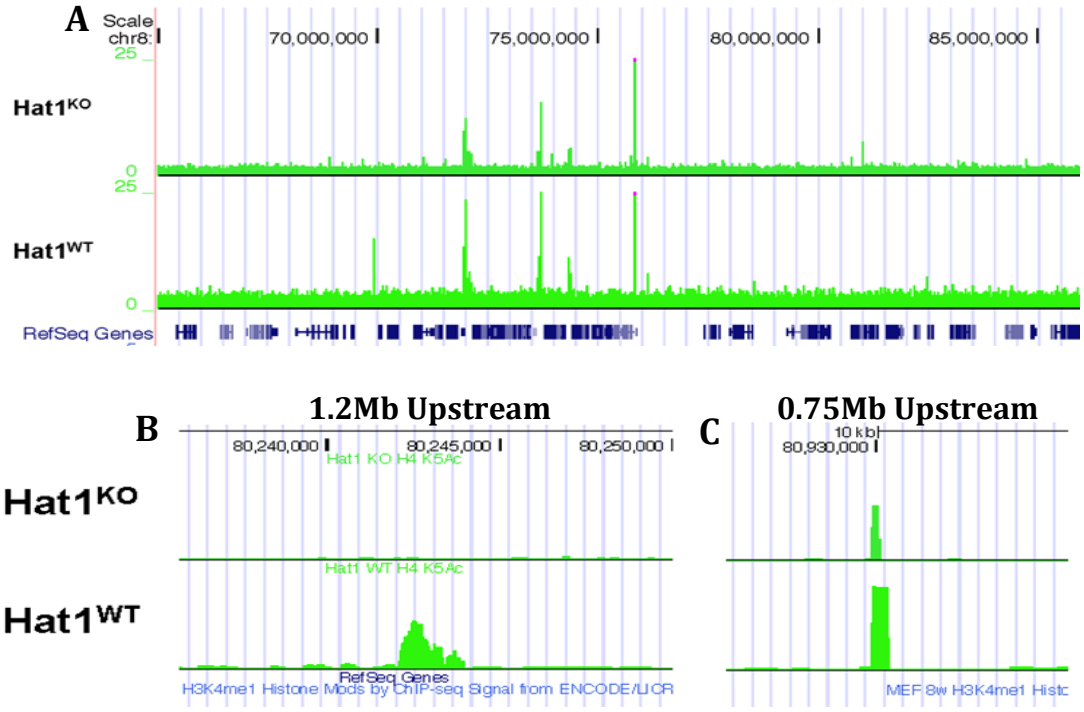


Figure 6. Global and locus-specific influence of Hat1 on the chromatin architecture[1].
Targeting of H4K5 acetylation on Hat1$^{+/+}$ (Hat1^{WT}) and Hat1$^{-/-}$ (Hat1^{KO}) chromatin in MEF cells by ChIP-Seq A) Globally, Hat1$^{-/-}$ MEF chromatin shows a uniform decrease in the baseline level of H4K5 acetylation. B) Locally, Hat1$^{-/-}$ MEF chromatin shows 853 locus-specific peaks of H4K5 acetylation that are either completely-dependent or C) partially-dependent upon the presence of Hat1. B) Image shows locus-specific peaks in H4K5 acetylation 1.2Mb and C) 0.75Mb upstream of the Lphn3 gene

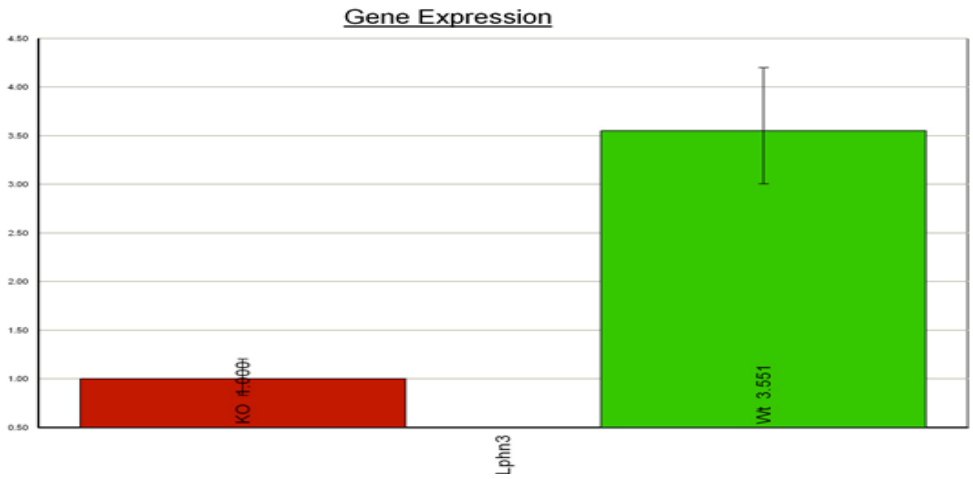
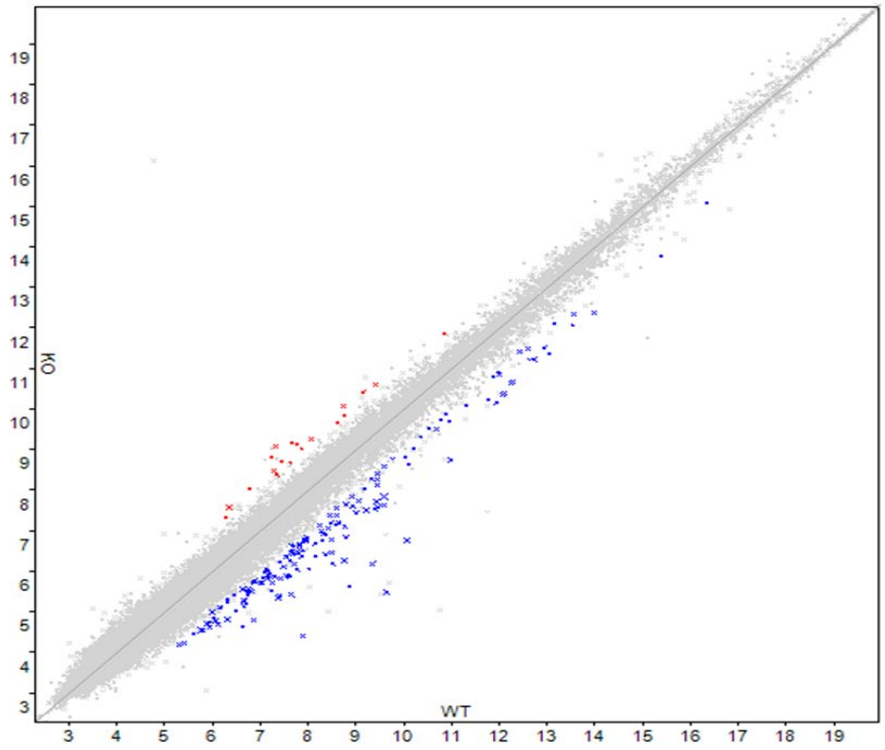


Figure 7. Hat1-dependent peaks in H4K5 acetylation influence gene expression.
Lphn3 shows peaks in H4K5 acetylation completely-dependent and partially-dependent on the presence of Hat1 upstream at 1.2Mb and 0.75Mb respectively. Hat1$^{-/-}$ MEFs show a 3.5-fold decrease in Lphn3 mRNA expression. Lphn3 gene expression was quantified by quantitative real-time PCR and normalized by GAPDH.

Figures

A



B

Gene	Fold Change in Expression (WT vs KO)
Ear2	-17.85
Xdh	-9.89
Ifi44	-9.40
Gdf10	-5.96
Mamld1	-5.00
Ly6c	-4.74
Stfpa1	-2.36
Cyp-2f2	-1.52

Figure 8. Hat1 gene expression profile in the embryonic lung.
Microarray data was analyzed in biological triplets of $Hat1^{+/+}$ and $Hat1^{-/-}$ embryonic lung tissue at E17.5. A) Microarray scatter plot for genes down-regulated at least 2-fold in the $Hat1^{-/-}$ lungs. There were 172 RNA transcripts that were found under-expressed at least 2-fold and 19 RNA transcripts that were found to be over-expressed at least 2-fold in the embryonic lungs of $Hat1^{-/-}$ embryos at E17.5. B) A selection of genes found to be under-expressed. Stfpa1 and Cyp-2f2 are markers for differentiated alveolar and Clara cells.

Figures

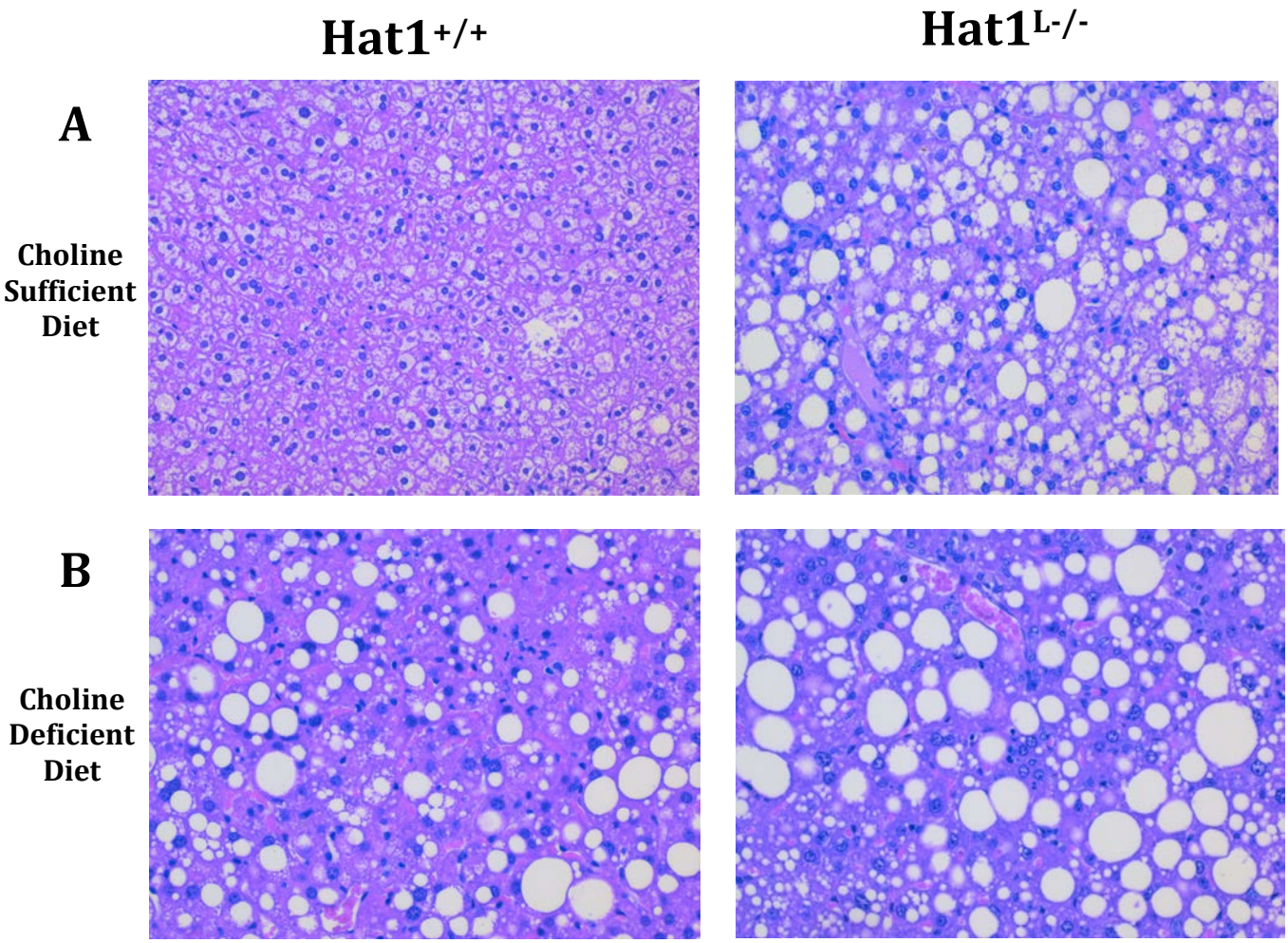


Figure 9. Loss of Hat1 results in an accumulation of fat in the liver resembling non-alcoholic fatty liver disease.
Pathological examination of 20 week old $Hat1^{+/+}$ and $Hat1^{-/-}$ mice fed on an A) Choline sufficient diet and B) Choline deficient diet. Choline deficient diets induce liver steatosis (an accumulation of fat in the liver) similar to what occurs during non-alcoholic fatty liver disease. Livers of $Hat1^{L-/-}$ mice fed on a choline sufficient diet resembles those of $Hat1^{+/+}$ mice fed on a choline deficient diet.

Figures

A

$Hat1^{+/+}$

B

$Hat1^{L-/-}$

C

Tumor Type	$Hat1^{+/-}$ (n=4)	$Hat1^{L-/-}$ (n=3)
Hepatocellular Carcinoma	**2**	**2**
Hepatoblastoma	**1**	**1**
Hepatocholangiocellular Carcinoma	**2**	**2**

Figure 10. Loss of Hat1 in the liver results in a high incidence of common and rare cancer types. A) Phenotypically normal liver from a $Hat1^{+/+}$ mouse at 95 weeks. B) Livers taken from mice with a liver-specific knockout of Hat1 at 80 weeks display multiple tumors. C) Tumor types represented amongst 80 week old mice containing liver-specific deletions of one ($Hat1^{+/-}$) or both alleles ($Hat1^{-/-}$) of Hat1. $Hat1^{+/-}$ mice include those that were completely heterozygous for Hat1 ($Hat1^{+/-}$) and those that were liver-specific heterozygous for Hat1 ($Hat1^{+/L-}$). There were no differences observed between the complete or liver-specific heterozygous mice.

Figures

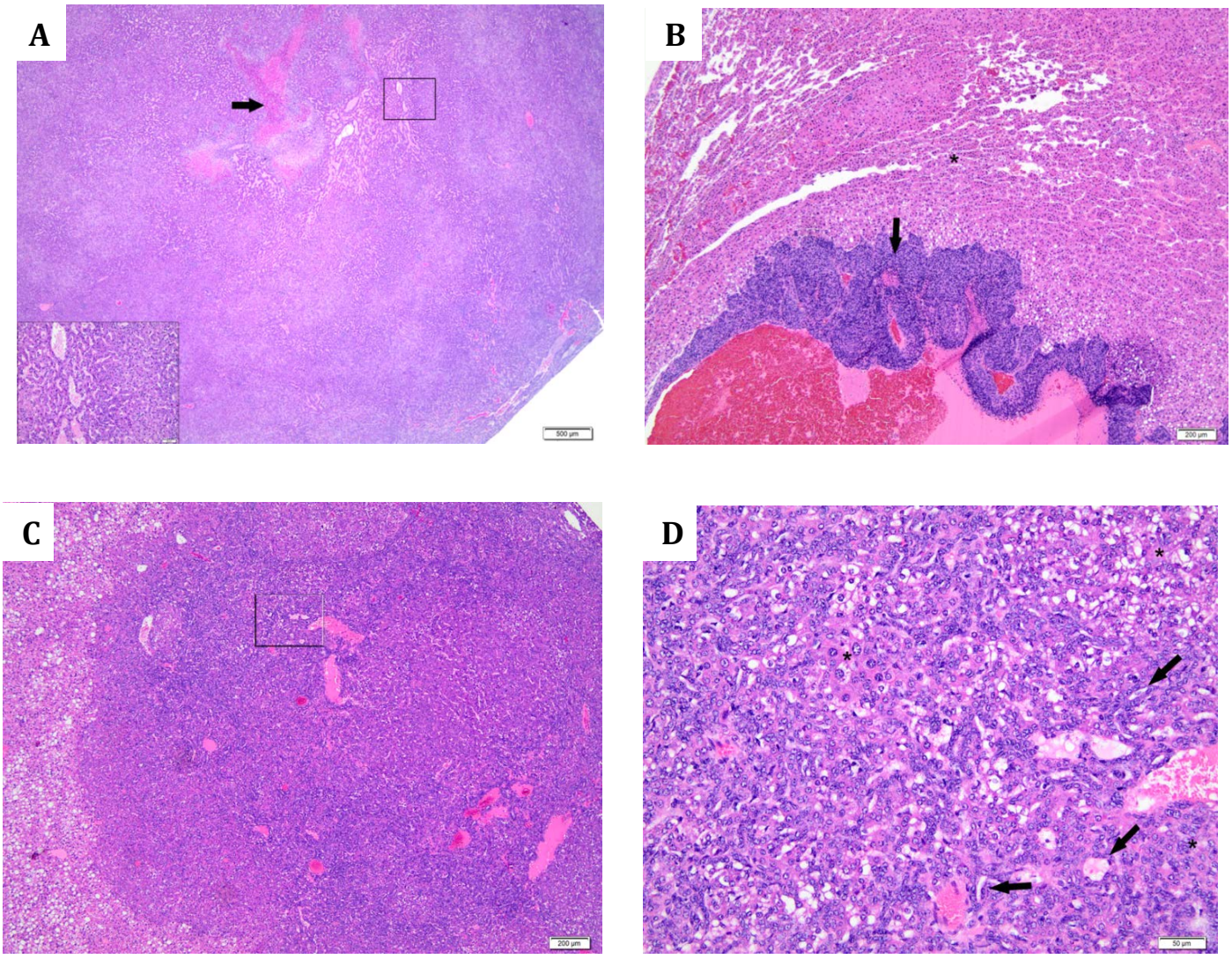


Figure 11. Histology of various cancer types found in 80-week old mice containing a liver-specific deletion of Hat1.
Histology of liver cancers found in 80-week old mice containing a deletion of Hat1. Loss of Hat1 in the liver leads to the formation of A) hepatocellular carcinoma, B) hepatoblastoma and C) hepatochonlangiocellular carcinoma. D) Higher magnification of hepatocholangiocellular carcinoma shows the formation of crude biliary structures (black arrows) in the livers of mice containing a deletion of Hat1.

Figures

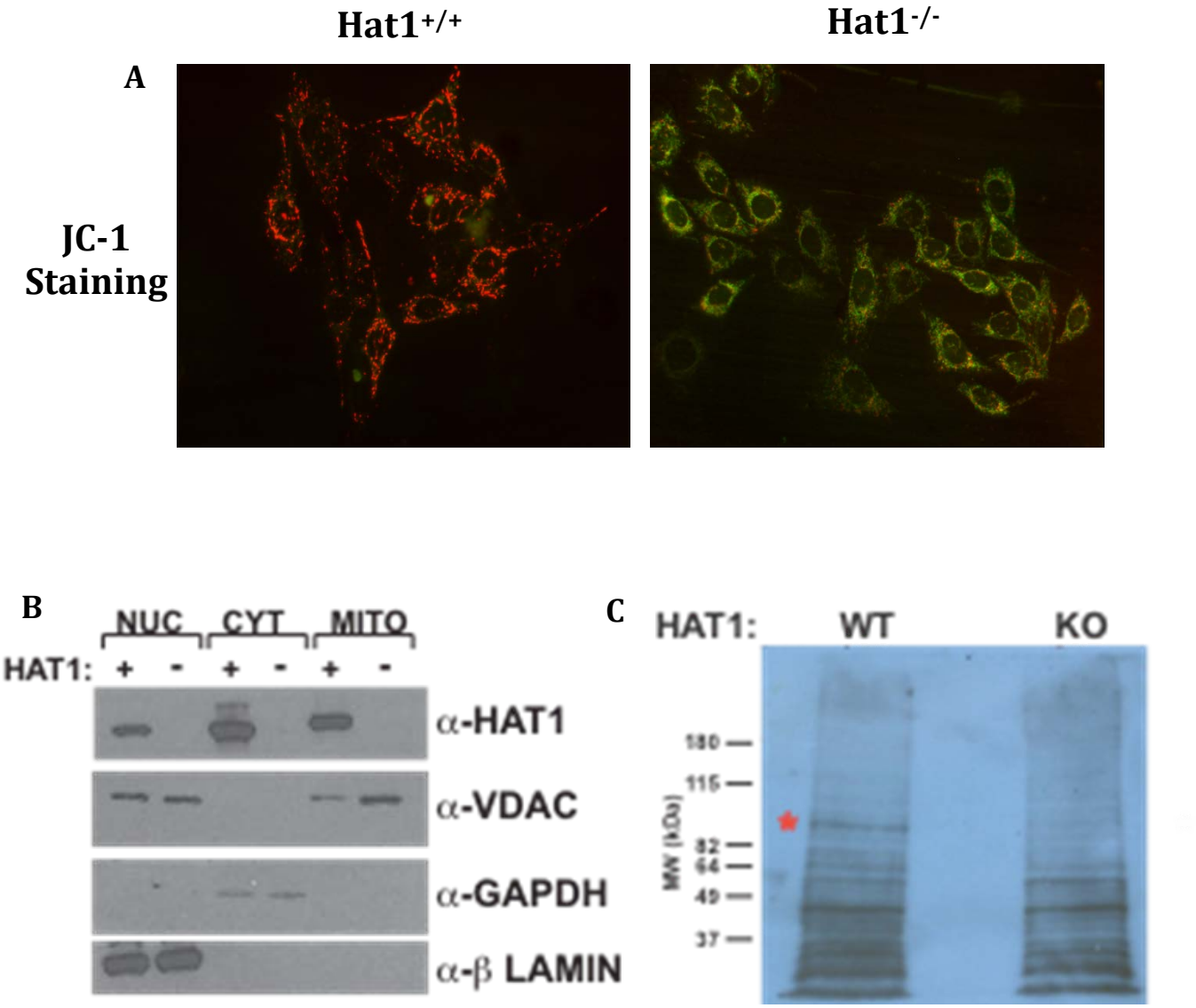


Figure 12. Hat1 may play a role in directing appropriate mitochondrial function by direct acetylation of mitochondrial proteins[3].
A) Hat1$^{+/+}$ and Hat1$^{-/-}$ MEF cells incubated with JC-1 indicates that Hat1$^{-/-}$ display defects in maintaining mitochondrial membrane potential, which is essential for oxidative phosphorylation. B) Hat1 localizes to the mitochondria and C) Hat1$^{-/-}$ MEFs show altered patterns of mitochondrial protein acetylation.

Figures

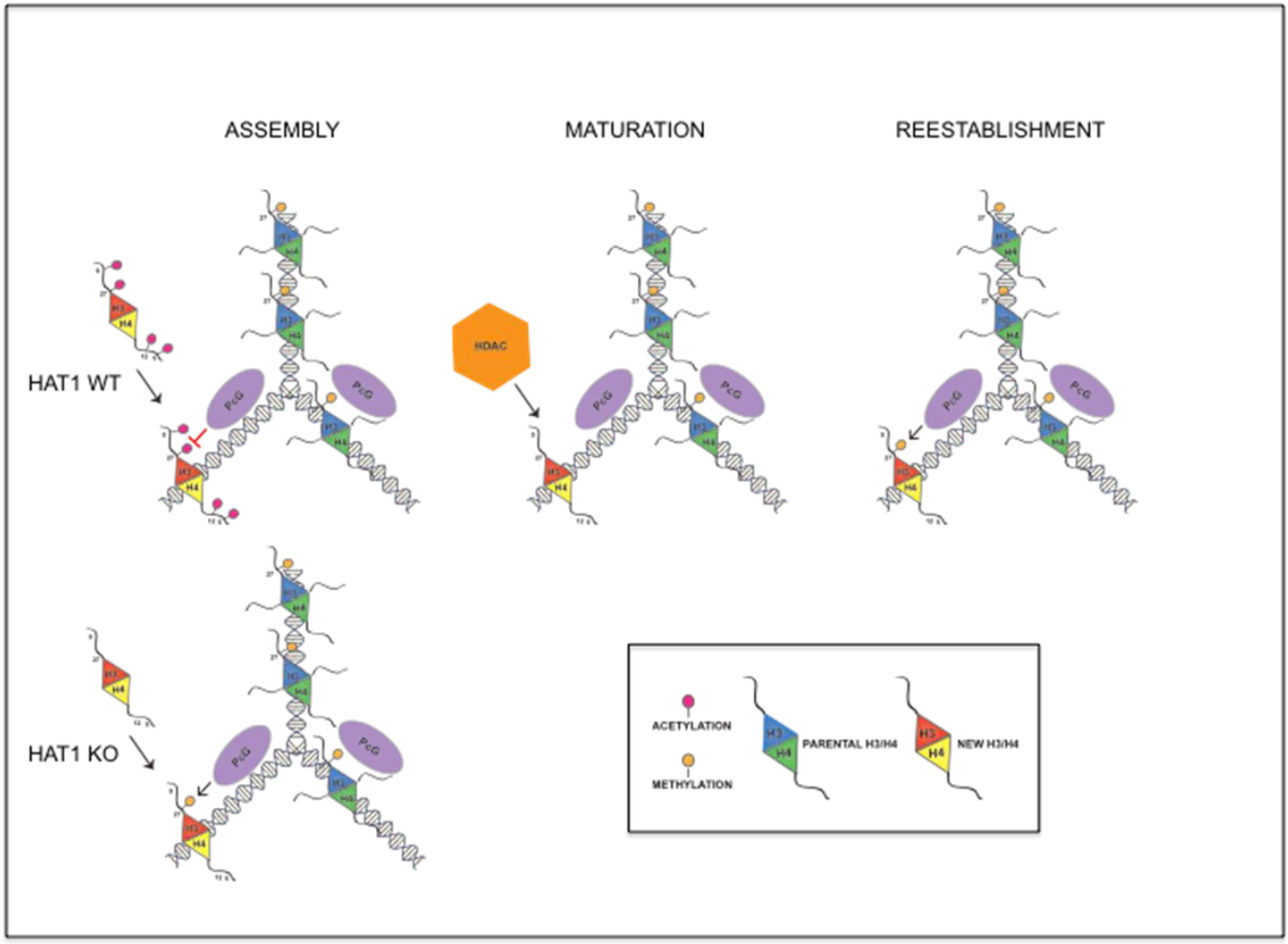


Figure 13. Proposed model for the essential role of Hat1 in epigenetic inheritance. Hat1 acetylation of Histone 3 at lysines 9 and 27 prevents the rapid trimethylation of lysine 27 by the Polycomb Group (PcG) during replication-coupled chromatin assembly. During maturation, these histone acetylation marks can be removed by HDACs to allow PcG to re-establish trimethylation of lysine 27. In the absence of Hat1, PcG rapidly re-establishes trimethylation of lysine 27 prior to chromatin maturation. PcG typically directs repressive trimethylation patterns at genes that induce cellular differentiation. Under our proposed model, loss of Hat1 would prevent the activation of cellular differentiation signaling pathways necessary for appropriate mammalian development.

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Footnotes

1. ChIP-Seq experiments were performed by Dr. Prabakaran Nagarajan in the Parthun laboratory.

2. Before continuing into a discussion of my research, there are a few things that I feel should be noted. It is important to understand that the evidence I am providing here is preliminary and should not be considered sufficient to confirm or deny some of the major conclusions I will present. While we hope to have the evidence to dispute many of our concerns in the future, though you should expect critical elements for my conclusions to be omitted.

3. Experiments analyzing the influence of Hat1 on mitochondria were performed by Paula Agudelo-Garcia in the Parthun laboratory.